lation or surgical removal of the thyroid gland. Following 2 months on a thyroid suppressive diet, the extent of neointimal regrowth was compared 14 days after balloon injury in hypothyroid rats (Group 1, n = 8, thyroid stimulating hormone (TSH) = 9.6 micro IU/ml) and animals maintained on a normal diet (Group 2, n = 8). Neointima formation was significantly attenuated in Group 1: intima/medial ratios were 0.904 ± 0.118 mm^2 and 1.361 ± 0.061 mm^2 in Groups 1 and 2 respectively, ($p < 0.02$). Additional experiments in rats following thyroidectomy (n = 3) revealed a similar significant decrease in neointima formation following balloon injury. This inhibitory effect of hypothyroidism was maintained in animals who were also made hypercholesterolemic (Group 3, n = 8, TSH = 10.2 micro IU/ml, total cholesterol = 752 mg/dl, low-density lipoprotein = 674 mg/dl): intima/medial ratios were 1.361 ± 0.061 mm^2 and 0.822 ± 0.078 mm^2, in Groups 2 and 3 respectively, $p < 0.02$. Prolonged (60 days) cholesterol feeding alone (Group 4, n = 8) as suspected, did not result in elevated cholesterol levels, but also did not result in significant differences in intimal thickness when compared to animals in Group 2. These results suggest that thyroid hormone may be an important co-mitogen for the proliferative response of the rat carotid artery to balloon injury and may help partially explain the growth inhibitory effect of hypophysectomy.

MONDAY PM

914-74 Estradiol 17β Protects Against Homocysteine-induced Vascular Injury in Rat Thoracic Aorta

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Hyperhomocysteinemia produces atherosclerotic lesions with characteristic endothelial desquamation and intimal smooth muscle cell (VSMC) proliferation. *In vitro,* homocysteine is cytotoxic to endothelial cells and recent observations suggest that it may directly stimulate VSMC proliferation. Estrogen, on the other hand, inhibits VSMC proliferation both *in vivo* and *in vitro.* We evaluated the effect of estradiol 17β ($E_2\beta$) on homocysteine-induced VSMC proliferation in arterial segments from the rat thoracic aorta. Segments were placed overnight in Dulbecco's Minimum Essential Medium, supplemented with gentamycin (25 μg/ml), glutamine (2 mM) and 0.4% fatal bovine serum, before incubation for 30 hours with DL-homocysteine thiolactone (0.1–5.0 mM). Radiolabelled thymidine uptake was assessed in intact and deendothelialized arterial segments, in presence of $E_2\beta$ (30 nM) or vehicle (1% ethanol). In deendothelialized segments homocysteine elicited a concentration-dependent increase in ^{3}H-thymidine uptake, expressed as cpm/mg protein. Thymidine uptake increased from a basal value of 8694 ± 1465 to 36338 ± 2025, at 5 mM homocysteine concentration ($p < 0.01$). Intact arterial segments showed a significantly attenuated response to homocysteine stimulation. On the other hand, incubation of deendothelialized segments with $E_2\beta$ (30 nM) caused a significant inhibition of homocysteine-stimulated response, without affecting basal ^{3}H-thymidine incorporation. These results provide evidence for a direct stimulatory effect of homocysteine on VSMC proliferation in rat aortic segments. Further, our data show estradiol 17β protects against homocysteine-induced vascular injury, possibly via a direct effect on VSMC.

914-75 The Role of Estradiol 17β on Free Radical Induced Vascular Myointimal Hyperplasia

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Oxygen free radicals may be involved in vascular hyperplasia by promoting smooth muscle cell proliferation, a response potentiated by endothelial damage. Estradiol 17β protects against vascular injury in experimental arteriosclerosis and inhibits proliferation of primary cultures of vascular smooth muscle cells. We studied the *in vitro* effect of estradiol 17β on vascular smooth muscle cell hyperplasia induced by the superoxide anions ($O_2^{-\bullet}$). Intact and denuded rat aortic segments were incubated overnight at 37° C in 2 ml Eagle's minimal essential medium supplemented with gentamycin and glutamine, without phenol red. The segments were challenged with either vehicle alone or the $O_2^{-\bullet}$ generating system (Xanthine and xanthine oxidase) for 4 hrs. We found that $O_2^{-\bullet}$ significantly enhanced ^{3}H-thymidine incorporation, an effect potentiated by the absence of the endothelium (Intact vessel 8.3 × 10^5 ± 0.1 × 10^5 vs denuded vessel 2.5 × 10^6 ± 1.3 × 10^5 $p < 0.01$). This effect was inhibited by superoxide dismutase. Estradiol 17β (30 nM) significantly inhibited free radical induced ^{3}H-thymidine uptake in both intact and denuded vessels. We conclude that an intact endothelium may protect against free radical induced smooth muscle cell proliferation. Estradiol 17β may also protect against free radical induced vascular proliferation by a mechanism independent of the endothelium.

915 Coronary Physiology

Monday, March 20, 1995, Noon–2:00 p.m.
Ernest N. Morial Convention Center, Hall E
Presentation Hour: Noon–1:00 p.m.

915-76 ATP-sensitive Potassium Channels Contribute to Reactive Hyperemia in Humans

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Activation of ATP-sensitive potassium channels (KATP) of vascular smooth muscle causes hyperpolarization of the cell membrane and vasodilation. The purposes of this study were 1) to determine whether KATP contribute to reactive hyperemia following ischemia in humans, and 2) whether adenosine, a metabolite present in ischemic muscle, activates KATP. Accordingly, we studied the effect of tolbutamide (1 mM), a KATP inhibitor, on reactive hyperemic forearm blood flow in 12 normal subjects. Forearm blood flow (FBF) was measured by venous occlusion plethysmography. Forearm ischemia was produced by inflating a blood pressure cuff to suprasystolic pressures for 5 minutes. Following cuff release FBF was measured during the reactive hyperemic phase for 5 minutes. After a re-equilibration period the above protocol was repeated during intra-brachial artery infusion of either tolbutamide 1 mM (n = 6) or vehicle (n = 6). Blood flow was plotted vs. time and the area under the curve was calculated after subtracting baseline flow. Tolbutamide did not significantly alter basal FBF (2.5 ± 0.6 to 2.3 ± 1.0 ml/100 ml/min), or peak reactive hyperemic FBF (21.9 ± 9.2 to 22.6 ± 7.2 ml/100 ml/min) (each p = ns). However, tolbutamide significantly attenuated total hyperemic flow repayment; (156 ± 44 vs. 114 ± 32 ml/100 ml, $p = 0.02$). Vehicle did not impair basal flow, peak reactive hyperemic flow or repayment. Tolbutamide 1 mM did not attenuate adenosine-induced(15–500 mM) increases in FBF (n = 6). These data indicate that KATP contribute to vasodilation during reactive hyperemia in humans. Activation of KATP is not mediated by adenosine.

915-77 Big ET-1-Infusion in Man Causes Only Renal ET-1-Release but Potent and Long-lasting Systemic, Pulmonary, Renal and Splanchnic Vasoconstriction

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Plasma endothelin-1, ET-1, and the precursor, big ET-1, are demonstrable in healthy man and increase during e.g. myocardial infarction (Miyauchi et al 1989). To study the vascular effects of big ET-1 infusion, and its possible conversion of ET-1, healthy subjects received big ET-1 in doses of 4–8 $pmol \cdot kg^{-1} \cdot min^{-1}$ intravenously for 20 minutes. Blood samples were taken from systemic arterial and pulmonary arterial as well as jugular, deep forearm, hepatic and renal vein catheters for determination of cardiac output, CO, splanchnic (ESBF) and renal (ERBF) blood flows (indicators: cardiogreen and PAH) forearm blood flow (FBF, pletysmography), and big ET-1 and ET-1 like immuno-reactivity, (ET-1-Li). Big ET-1 infusion caused a decrease in heart rate, HR, from 57 ± 4 to 45 ± 3 $beats \cdot min^{-1}$ ($p < 0.001$) and increase of mean arterial pressure, MAP, from 86 ± 1.3 to 106 ± 3.2 mmHg ($p < 0.001$). CO fell by 22 ± 5% ($p < 0.01$). Pulmonary, splanchnic and renal oxygen uptakes were unchanged indicating that big ET-1 does not influence oxygen consumption. The arterial-jugular venous oxygen difference was unchanged indicating unchanged cerebral blood flow. FBF was unchanged. ERBF and ESBF fell by approximately 40% (both $p < 0.001$). Only splanchnic and renal tissues extract big ET-1 with fractional extractions of 23 ± 4 and 45 ± 4%. Intravenous infusion of big ET-1 increased arterial ET-1-Li from 4 to 8 $pmol \cdot l^{-1}$ ($p < 0.001$) and renal release of ET-1-Li from 1.50 ± 0.18 to 8.68 ± 0.64 $pmol \cdot min^{-1}$ ($p < 0.001$). *Conclusion:* Big ET-1 infusion causes falls in HR and CO, increase in MAP and decreases in splanchnic and renal blood flows. Uptakes of big ET-1 were only noted in the renal and splanchnic regions with two-fold higher values in the kidneys, $p < 0.005$. Only the kidney showed increased release of ET-1-Li suggesting a unique renal capacity to synthesize and release big ET-1. Compared to ET-1 infusion (Weitzberg et al 1991) big ET-1 infusion causes, despite lower arterial plasma ET-1-Li levels, more marked haemodynamic effects as reflected in drops in HR and CO and more marked increase in MAP with more pronounced and long-lasting renal vasoconstriction. In addition big-ET-1 had no effect on cerebral or skeletal muscle blood flows while ET-1 caused dilatations in these regions.